REMARKS/ARGUMENTS

Reconsideration of this application and entry of the foregoing amendments are respectfully requested.

At the outset, the Examiner is urged to reconsider and withdraw the finality of the present Action in view of newly cited art (Cullis et al). The term "extracellular" was introduced into claim 42 in the prior Amendment and, as pointed out there (see page 9), incorporation of the term clearly distinguished the invention over Smith et al. Should the Examiner have viewed Cullis et al to be relevant, it could have been and should have been cited previously. Applicants' prior Amendment in no way necessitated the new ground of rejection.

Claims 42, 64 and 69 have been revised so as to be drawn to an "*in vitro* or *ex vivo*" method. Basis for "*ex vivo*" can be found, for example, at page 13, line 11. Basis for "*in vitro*" is found, for example, in the numerous references in the specification to techniques to be carried out "*in vitro*". In this regard, attention is directed to, for example, page 13, line 9 (inanimate sample, such as effluent, water or foodstuff), page 14, lines 13-14 (foodstuffs, water samples), page 15, line 21 (water source, foodstuff), page 15, lines 24-25 (reference to analysis on a gel-like substrate), page 21, lines 17-19 (monitoring change in colour on a culture plate), page 35, lines 11-12 (agar plate assay for detecting bacteria in a sample), heading 1.5 (detection of bacteria in foodstuffs), and page 42, heading 2.2 (analysis in plastics cells). That the claims have been revised should not be taken as an indication that Applicants agree with any position taken by the Examiner. Rather, the revisions are offered merely to advance prosecution and Applicants reserve the right to pursue any deleted subject matter in a continuation application.

Claims 42, 45-47, 51, 52, 54, 58, 61 and 64-66 stand rejected under 35 USC 103 as allegedly being obvious over Cullis et al in view of Smith et al in light of Subbarao et al. Withdrawal of the rejection is in order for the reasons that follow.

The Examiner contends that newly cited Cullis et al discloses the use of lipid vesicle particles incorporating a cytolytic peptide which, in response to a predetermined extracellular metabolic signal, acts to mediate the opening of pores. In Cullis et al, the cytolytic peptide (e.g., GALA) is covalently bonded to the lipid layer. For the purposes of establishing an obviousness argument, the Examiner relies on Smith et al, in which the cytolytic peptide can be covalently or non-covalently located in the lipid layer (column 6, lines 46-48) to argue that it would have been obvious to modify Cullis et al to use cytolytic peptides that are non-covalently bound within the lipid layer.

The Examiner also relies on Subbarao et al which discloses lipids incorporating non-covalently bound cytolytic peptides. Subbarao et al discloses liposomes that incorporate GALA (a cytoytic peptide) in the lipid bilayer. Subbarao et al relates to an investigation of the rôle of proteins in the control of biological membrane fussions. More particularly, Subbarao et al is a study of bilayer stability in the presence of the cytolytic peptide and concludes that the liposomes are "stable" at pH about 7 but "leak" at pH about 5.5.

The Examiner's position appears to be that Smith et al teaches the "equivalence" of covalently and non-covalently bonded GALA in liposomes, Subbarao et al effectively confirms that non-covalently bonded GALA works, therefore, it would have been obvious to use non-covalently bonded GALA in the approach described by Cullis et al. Applicants respectfully disagree.

Claim 42 is drawn to a method of detecting a cell type of interest present or potentially present in a sample. In essence, this is an "analytical method" and not a drug delivery technique, such as contemplated in the Cullis et al and Smith et al. In order to make this distinction additionally clear, the independent claims as now presented explicitly state that the method is an "*in vitro* or *ex vivo*" method.

In the prior Office Action, Smith et al was cited as the primary reference. As Applicants understand it, Smith et al was withdrawn as the primary citation because it did not disclose liposomes that responded to an "extracellular" metabolic signal (as noted above, the term "extracellular" was introduced into claim 42 in the prior Amendment). It appears that the Examiner now cites Cullis et al on the basis that, in his view, it discloses the method of claim 42 (including the "extracellular metabolic signal" limitation) but for the fact that in the Cullis et al lipid vesicle particles (liposomes), the cytolytic peptide is covalently attached to the enveloping lipids rather than non-covalently as required by claim 42. To cure this "deficiency" the Examiner relies on Smith et al since it discloses liposomes with a cytolytic peptide non-covalently incorporate the lipids.

Applicants direct the Examiner's attention to the fact that there are actually a number of distinctions between the present invention and the teachings of Cullis et al.

Firstly, the foregoing revision of the claims to recite an "*in vitro*" or "*ex vivo*" method makes clearer the distinction over the drug delivery technique of Cullis et al.

Secondly, Cullis et al relates to fusogenic liposomes for the purpose of drug delivery. Essentially, these liposomes fuse to a target site for the purposes of delivering a drug carried by the liposome (see column 2, line 45 to column 3, line 30). In particular, it should be noted that the paragraph at column 3, line 13 makes reference to the "lipopeptide" (at the beginning of the

paragraph) and reference at the end of the paragraph (incorporating the lipopeptide) to its being stable at physiological pH but becoming destabilized and fusing with the endosome membrane on exposure to an acidic pH. Further information with regard to the lipopeptide is provided in the section beginning at column 16, line 23. In that section, the "fusogenic lipopeptide" is defined as comprising a lipid **covalently** attached to a peptide by means of a covalent bond. Incorporation of the lipopeptide into the liposome enhances the fusogenic properties of the latter (column 16, lines 39-41). Further description of peptides used to form the lipopeptide is given in the paragraph beginning at column 16, line 58. There are a number of references, one of which refers to GALA (column 17, lines 32-33) (the sequence listing at column 17, lines 42-43, includes specific reference to GALA).

The point to be made is that the lipopeptide in Cullis et al comprising the covalently bonded peptide (with the latter possibly being GALA) is provided to enhance the fusogenic properties of the liposome (column 16, lines 39-41) although the two may not be unrelated rather than modulating permeability (see column 25, lines 11-14).

Moreover, reference is made to the disclosure at column 57, lines 38-60, in particular, the final sentence relating to the difference in behavior for the peptide in (AcE4K – SEQ ID NO:3) and in its form when covalently coupled to a lipid. This would suggest that the Examiner's assumed equivalence of the free and covalently-bound peptide may not be well founded.

This is a different purpose from the use of GALA in the lipid vesicle particles of the present invention. In these particles, the cytolytic peptide (e.g., GALA) is non-covalently incorporated in the lipid by-layer and is not provided to enhance fusogenic properties of the lipsomes but rather to modulate permeability in response to a metabolic signal.

In both Smith et al and Cullis et al, the desired interaction of the liposome with its target, under conditions of lowered pH, is membrane fusion such that the contents of the liposome are delivered to the interior of the target cell. Cullis et al developed assays to distinguish effects of membrane destabilization between the desired (membrane fusion) and the unwelcome (content leakage).

In the passage that the Examiner refers to (column 31, lines 44-45), Cullis et al teaches that:

> "Another approach has used liposomes prepared from pH-sensitive lipids, which leak their pharmaceutical contents into low pH target regions. Such areas of localized acidity are sometimes found in tumors"

The requirement of the claimed invention that the lipid vesicle particles be targeted to a targeted cell type is clearly distinguishable from the cited art in that the cytolytic peptide responds to a metabolic signal from the cell to which it is targeted to bring about the generation of a detectable signal. In Cullis et al, it is suggested that liposomes targeted to tumor cells may be destabilized by the localized acidity sometimes found in tumors. It does not follow that the acidity is a metabolic signal from the cell to which the liposome is targeted. This acidity is a consequence of oxygen starvation of cells of all types, within, and around, a tumorous mass which does not have adequate blood supply. It follows that any type of cell which is so starved of oxygen will contribute to this localized acidity.

Since the liposomes of the claimed invention respond to a metabolic signal from the target cell, it follows that the cell is metabolically active and is, therefore, a 'living' cell. By responding to a metabolic signal, the method of the present invention can differentiate between living/active and dead/inactive cells (or, at least, it detects living but not dead cells). This

contrasts starkly with other techniques for rapid detection of organisms, for instance, by detecting specific DNA sequences (e.g., PCR tests) or proteins (e.g., immunoassays) which cannot differentiate living from dead cells within the sample.

It also contrasts with the teaching of Cullis et al since the liposome of Cullis et al is responding to 'localized acidity' at the site of the tumor. It may well be that the Cullis liposomes are targeted to dead or metabolically inactive cells within the area of localized acidity and will, thus, falsely identify a dead cell. Equally, the target cell may be in an area of better blood supply, within the area of localized acidity, and may not be generating the metabolic signal (acidity).

The Examiner is urged to give careful consideration to the foregoing comments. It is believed that, having done so, the Examiner will find withdrawal of the rejection to be in order.

Claims 48-50 stand rejected under 35 USC 103 as allegedly being obvious over Cullis et al in view of Smith et al in light of Subbararo et al and further in view of Bally et al. Withdrawal of the rejection is submitted to be in order for the reasons that follow.

Bally et al discloses liposomes as summarized at column 2, lines 31-54. The liposomes may be loaded with a bioactive agent (column, lines 36-38) and used for drug delivery purposes. To this extent, the liposomes of Bally et al are different from those of the present invention and are used for a different purpose. Bally et al discloses that the liposomes may be used in diagnostic assays (column 3, lines 44-48). There is reference to aggregation at column 5, lines 17-30, which (it is stated) may be exploited in aggregation-type diagnostic assays. However, what the passage in column 5 appears to primarily be disclosing is that certain methods for production of the Bally et al liposomes involve contacting biotinylated PE (phosphatidylethanolamine – see column 3, line 54) with streptavadin. The streptavadin incorporates four biotin binding sites. If there is excess biotinylated PE, it will bind to more than

one biotin binding site on the streptavadin thereby giving rise to **unwanted** aggregation in the manufacture of the liposomes. The subsequent reference to the aggregation phenomenon being exploited in an aggregation-type diagnostic assay is merely a passing reference without further explanation.

Applicants respectfully submit that Cullis et al is utterly irrelevant to the subject matter of claim 48 and for the Examiner to contend otherwise underscores the fact that the rejection is based on improper hindsight reasoning.

Cullis et al does not relate to:

(i) an *in vitro/ex vivo* assay,

(ii) detecting metabolically active cells, or

(iii) modifying the liposomes so that they are targeted to, and aggregate around, the cells of interest.

Withdrawal of the rejection of claims 48-50 is clearly in order and the same is requested.

Claims 55-57 stand rejected under 35 USC 103 as allegedly being obvious over Cullis et al, Smith et al, Subbarao et al in view of Levinson et al. The failings of Cullis et al, Smith et al and Subbarao et al, are discussed in detail above. Nothing in Levinson et al would have cured those deficiencies. It is submitted that this rejection is based on hindsight and reconsideration is requested.

Claims 59 and 69 stand rejected under 35 USC 103 as allegedly being obvious over Cullis et al, Smith et al, Subbarao et al as supplemented by Robinson et al. Applicants see no justification for the Examiner's position that it would have been obvious to detect pathogenic cells in foodstuffs in the Cullis et al in order to allow for the rapid identification of diseases that are effecting plant crops. Cullis et al is concerned with drug delivery. It has absolutely nothing

to do with analytical procedures for foodstuffs. It is only with hindsight that the rejection could have been made. Reconsideration is requested.

Claim 60 stands rejected under 35 USC 103 as allegedly being obvious over Cullis et al, Smith et al, Subbarao et al in view of Blondin et al. The failings of Cullis et al, as well as Smith et al and Subbarao et al, are discussed in detail above. Nothing in Blondin et al would have cured those deficiencies. It is submitted that this rejection, like those above, is based on hindsight and reconsideration is requested.

This application is submitted to be in condition for allowance and a Notice to that effect is requested.

Respectfully submitted,

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